Running title: Xeroderma Pigmentosum in Finland

NEUROLOGICAL SYMPTOMS AND NATURAL COURSE OF XERODERMA PIGMENTOSUM

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Summary

We have prospectively followed 16 Finnish Xeroderma pigmentosum (XP) patients up to 23 years. Seven patients were assigned by complementation analysis to the group XP-A, two patients to the XP-C group and one patient to the XP-G group. Six of the seven XP-A patients had the identical mutation (Arg228Ter). The seventh patient has a different mutation (G283A). Further patients were assigned to complementation groups on the basis of their consanguinity to a XP patient with a known complementation group. The first sign of the disease in all the cases was severe sunburn with minimal sun exposure in early infancy. However, the diagnosis was made at that time only in two cases. The XP-A patients developed neurological and cognitive dysfunction already in childhood. The neurological disease advanced in orderly fashion through its successive stages, finally affecting the whole nervous system and leading to death before the age of 40 years. Dermatological and ocular damage of the XP-A patients tended to be limited. The two XP-C patients were neurologically and cognitively intact in spite of the mild brain atrophy in neuroimaging. The XP-G patients had sensorineural hearing loss, laryngeal dystonia and peripheral neuropathy. The XP-C patients had severe skin and ocular malignancies first presented at preschool age. They also showed immunosuppression in cell-mediated immunity. Neurological disease appears to be associated with complementation group and the failure of fibroblasts to recover RNA synthesis following UV irradiation, but not necessarily to the severity of the dermatological symptoms, the hypersensitivity of fibroblasts to UVB killing, or the susceptibility of keratinocytes to UVB-induced apoptosis.

Keywords

Xeroderma pigmentosum, neurodegeneration, ultraviolet, cancer, complementation group

Introduction

Xeroderma pigmentosum is a rare autosomal recessive disease with cutaneous, ocular and neurological symptoms. The basic defect is in DNA repair. Xeroderma pigmentosum was first described clinically in 1874 by Hebra and Kaposi (Hebra and Kaposi, 1874; Kaposi, 1882) as a syndrome of sunlight hypersensitivity, freckles and skin cancers. De Sanctis and Caccione (1932) reported the neurological manifestations of the disease in 1932. XP occurs in all races. The frequency is approximately 1: 250,000 in Europe and USA, in Japan it is higher, 1: 30,000 (Kraemer et al., 1987).

XP patients have been assigned into eight complementation groups. Seven of these (XP-A to XP-G) are associated with defects in nucleotide excision repair (NER), the process in which bulky lesions including UV photoproducts are removed from the DNA. In the eighth form (XP variant form, XP-V) the defect is in translesion synthesis, the ability to replicate DNA templates carrying unrepaired DNA damage. All the genes and chromosomal locations associated with the complementation groups have been identified (Wattendorf and Kraemer, 2005). XP-A and XP-C are the most common complementation groups in Europe. In general, XP-A patients have the most profound DNA repair defect with minimal or no repair activity and in this group neurological symptoms are common. In XP-C the skin problems are severe, but neurological symptoms are rare. Neurological, dermatological and ocular symptoms may vary from mild to severe in two persons with the same complementation group, or even sibs (Kraemer and Slor, 1985).

The disease typically starts with skin symptoms. Progressive neurological manifestations, including cognitive deterioration, occur in about 20-30% of the patients, more commonly in groups XP-A, D and G. Ocular symptoms are seen in nearly 80 % of XP patients starting early in childhood with photophobia and conjunctivitis. Later malignant tumours may also involve the eyes.

In the present study, we have prospectively followed neurological abnormalities and other clinical manifestations in 16 Finnish XP-patients for up to 23 years. Neuropathology of one of our patients (XP-A) has been published earlier (Roytta and Anttinen, 1986).

Patients and methods

We have prospectively followed the clinical course of 16 Finnish XP patients from 2 to 23 years. This is a hospital based patient study from different departments of dermatology and neurology in Finland. The follow up started in 1981 and so far 16 patients have been studied. The patients were given a case number in the order they were included into the study. The examinations were carried out in Turku University Central Hospital. Most patients were fully examined twice (1982, and 1988 or 1998) as in-patients and more often followed up in the dermatological and neurological out-patient clinics. The first clinical examination took place between the age of one and 36 years. Each subject or his/her guardian was fully informed of the procedures and gave a written informed consent before the entry into the study, which was approved by the local Ethics Committee.

The patients have been examined clinically by a neurologist, dermatologist and neuro-ophthalmologist. In addition, a computed tomography of the brain or /and a brain MRI (1,5 Tesla) were performed where applicable. The otological examination included an audiogram. Standard EEG-recordings were obtained and electroneuromyography (ENMG) examination and interpretation were made by the clinical neurophysiologist. Neuropsychological examinations were carried out to measure verbal, visuomotor and memory performances. The overall level of performance was evaluated using three verbal (Arithmetic, Similarities and Digit span) and four visuomotor (Digit symbol, Picture completion, Block design and Object assembly) subtests of the WAIS (Wechsler 1955). Digit span forwards and backwards served as a measure of working memory.

Since the T cell-mediated tumor immunity is a major defence strategy against neoplasms, we studied the cell-mediated immune reactivity in XP patients using the contact hypersensitivity (CHS) model (Cooper et al., 1992).

Skin punch biopsies were obtained from ten cases and fibroblast cultures established according to standard procedures and ethical guidelines. For five cases, it was also possible to establish short-term keratinocyte cultures (Petit-Frère et al.,2000). Cellular DNA repair assays were performed in the

former MRC Cell Mutation Unit (now the Genome Damage and Stability Centre), University of Sussex, Brighton and in the Erasmus University. UVB survival (Arlett et al., 1992), UVC survival (Jaspers et al., 2002), unscheduled DNA synthesis (Lehmann and Stevens, 1980) and (Vermeulen et al., 1991) and long-term inhibition of RNA synthesis in fibroblasts (Lehmann et al., 1993) were determined as described previously. Complementation analysis to identify the affected gene was performed in The Medical Genetic Cluster, Erasmus University in Rotterdam as described previously (Vermeulen et al., 1991). Apoptosis and cytokine release and expression in keratinocytes have been reported previously (Petit-Frère et al., 2000). The mutations in the XP-A patients were determined using RT-PCR from total RNA and direct sequencing of the amplified cDNA.

The XP-G patients were examined only clinically in the year 2002. One of them had been diagnosed prenatally.

Results

Complementation analysis

Complementation analysis has been carried out in nine cases since 1996. At that time five patients were already deceased. Seven of those analyzed turned out to be XP-A (definite XP-A), two patients were XP-C and one, earlier, prenatally diagnosed, XP-G (Table 1). Cases 1 and 3 were sisters of Case 11, whose complementation group was XP-A. The healthy mother of XP-A case 14 is a cousin of case 9. The XP-G patients are brothers. Complementation analysis was not carried out for cases 2 and 4. The mutations in the XP-A patients were identified by sequence analysis. Cases 5, 7, 11-14 had the identical mutation a homozygous truncation mutation at codon 228 (Arg228Ter) in the last exon of the XPA protein. Case 6 has the homozygous mutation G283A, changing the last base of exon 2. This results in the majority of the mRNA being aberrantly spliced with deletion of one or more of the exons downstream of exon 2.(Table 6)

Dermatological signs

According to the clinical records, the first clinical sign in all the cases was severe sunburn with blistering or persistent erythema after minimal sun exposure, like sleeping early in spring in a shaded place in a pram. This occurred in all the cases (XP-A, XP-C, XP-G) before the age of one, but led to diagnosis in only two of the cases (9 and 14). Later the sensitivity to sunburn diminished in some of the patients. In all the patients except case 14 freckling of the face was evident at the age of two years.

At clinical examination, poikiloderma with hyper- and hypopigmentation and skin atrophy was seen in all the patients except case 14, who has been carefully protected from UV-exposure. Absence of pigmentary changes was evident on the areas protected from sunlight (Fig.1).

The first skin malignancies were diagnosed in the XP-C patients at preschool age. As a total, both of them have had over hundred non-melanoma skin cancers (NMSC). In the XP-A patients the malignancies occurred later, and half of the XP-A patients have had no skin malignancies. One of the XP-A patients has had a cutaneous malignant melanoma (CMM) and one of the XP-C cases has had several CMMs. The XP-G patients have had no skin malignancies.

For seven cases (5 XP-A, 2 XP-C) the basal cutaneous cell-mediated immune reactivity to diphenylcyclopropenone (DPCP) was investigated using a DPCP sensitization dose which sensitizes every healthy control person. A primary allergic response (PAR) is an indicator of aroused sensitization. It is a spontaneous flare of contact hypersensitivity at the site of allergen application 1-2 weeks after the epicutaneous application of the allergen. Later the sensitized individuals react when challenged to small amounts of the same allergen. Three of the patients showed basic immunosuppression in cell-mediated immunity, they were not sensitized to DPCP (Table 2, both the XP-C cases and XP-A Case 6). Basic immunosuppression of cell-mediated immunity is likely to contribute to the development of skin malignancies especially in XP-C patients

Ophthalmological signs

Eleven out of the thirteen patients who underwent ophthalmological examination had eye symptoms. Common complaints were photophobia, excessive weeping, blepharospasm and/or decreased vision.

Seven patients had irritative conjunctivitis and nine pterygia.

Four patients had nodular tumours in their eye lids. One patient had limbal premalignant dysplasia. Symblepharon (Fig.2) was observed in six patients; two of these patients had experienced several oculoplastic surgeries.

Keratopathy, scarring, exposure keratitis and premalignant dysplasia were corneal findings in five patients and progressed to permanently severe visual loss in two eyes despite oculoplastic surgery and penetrating keratoplasty. Examples of the ophthalmological findings are illustrated in Fig.2.

Eye examinations did not reveal neuro-ophthalmological pathology.

The visual prognosis was poor in two patients with XP-C. One patient became permanently blind due to the malignant eye lid, conjunctival and corneal changes. The other patient lost the right eye, because of severe symblepharon and corneal ulcerations.

Neurological signs

The clinical course of the disease in definite and probable XP-As is presented in Tables 1 and 3 With one exception (Case 6, late juvenile onset type) the neurological manifestations and the clinical course of the disease are similar. All these patients have short stature and their heads are microcephalic. Secondary sexual characteristics are poorly developed. Case 6 has given birth to two sons, however.

The patients had normal early development until the age of two years. The first neurological symptoms appeared before the age of 8 years. According to the development history given by the parents the first symptom was mild cognitive impairment in all the cases. The patients usually started school normally. However they did not manage a normal school and had to continue approximately within three years in special schools for mentally retarded children. All the patients showed slight to severe diffuse encephalopathy in EEG.

The next symptoms were cerebellar, predominantly presenting as dysarthria without swallowing

problems. Difficulties in speaking were followed by disturbances in balance. The neurological signs were ataxia of the legs with milder ataxia of the upper limbs. Cerebellar symptoms usually occurred between the age of 4-16 years. Later, progressive difficulties in walking are explained also by concurrent neuropathy. The clinical examination revealed areflexia and neuropathy was confirmed by ENMG, which showed moderate to marked axonal sensory motor neuropathy. Neuropathy was usually first seen in the second decade, becoming more severe in the third decade. (Table 3)

In addition to cognitive impairment, the patients also exhibited an unusual mental symptom occurring as increased sensitivity and tendency to weep and to be frightened. This symptom was common (8 / 11 adults). Six out of the 11 adult patients had developed choreoathetoid type involuntary movements, predominantly in the upper limbs, starting in early adulthood.

Sensorineural deafness in audiogram was seen in all cases, except Case 6.

Corticospinal involvement was usually observed in the third decade, slowly progressing to spastic tetraplegia some years before the death. At that time the cognitive impairment was severe and the patients needed help in all daily activities. They had severe difficulties in swallowing and walking, needed a wheelchair and were bedridden within a couple of years.

Seven patients have died, all severely neurologically affected. The median age at death was 33 years (29-40yr). The cause of the death was pneumonia in all the cases.

The XP-C patients went through all the neurological examinations with normal findings.

The XP-G patients were examined once at the ages of 22 and 34 years. They are remarkably short in stature and have a bird like face (Fig.3). Both of them have severe laryngeal dystonia. They graduated from high school and are employed. Case 16 has cerebral palsy, spastic diplegia. Both cases have sensorineural hearing loss. Neurological examination revealed areflexia suggesting polyneuropathy. No ENMG examinations were carried out on these patients. Otherwise the neurological examination was normal.

Neuropsychological findings

Altogether 11 patients completed cognitive testing by a neuropsychologist. Four of the patients (2 XP-A and 2 XP-C patients) were examined twice within 15 years.

The two XP-C patients performed normally on all tasks both at initial examination and at follow-up 15 years later.

All but one (the late juvenile onset type, case 6) of the XP-A patients showed moderate to severe overall cognitive impairment (Table 4). The patient with late juvenile onset type performed otherwise normally at baseline except mild working memory difficulties, and showed further impairment in working memory and arithmetic but preserved other skills at follow-up 15 years later. Most XP-A patients had problems in speech production, not only due to dysarthria but also verbal processing deficits. There were no specific cognitive impairment profiles, but many patients could perform at a relatively good level in some cognitive domains, most often in semantic knowledge, in spite of overall impairment. (Table 4)

Neuroimaging

Nine XP-A patients and two XP-C patients were studied. Six patients (4 XP-A and 2 X-PC) were studied by 1.5 Tesla MRI, in case 6 the imaging was performed twice. At least T2-and T1-weighted images were obtained in all the cases. Six of the patients were studied by CT, in case 5 CT was performed two times. The summary of the findings is shown in Table 5.

Calvarial thickening was seen in 9 XP-A cases, in all of them the thickening was most prominent in frontal bones, the occipital bone was thickened only in 2 cases. Additionally, thickening of sphenoidal and temporal bones was observed in 3 cases.

Brain atrophy was demonstrated in all 11 studied patients, including the XP-C patients. In both the XP-C patients and case 6 (late juvenile onset type) general atrophy was mild while in other 8 patients the atrophy was moderate or severe. The atrophy was generally symmetrical and covered all lobes, deep

grey and white matter structures, brainstem and cerebellum. Of the patients with cognitive and memory impairment, general and hippocampal atrophy was light in case 6, and in the other cases moderate or severe.

In six patients including the XP-C cases white matter T2-hyperintensities were demonstrated with MRI. In 3 of them only slight periventricular changes and a hyperintense band in the capsula externa were detected. In 2 cases more advanced white matter pathology was seen; in cerebrum only U-fibres showed normal signal intensity. In one patient also superficial U-fibres were T2-hyperintense. In this case also hypointensity of putamen and nucleus caudatus was demonstrated on both sides Fig. 4.

Cellular studies

The experiments on fibroblast cultures are summarised in Table 6. Sensitivity to UVB killing was determined for four XP-As (Cases 5, 6, 12 and 13) and two XP-Cs (Cases 8 and 10). A Westinghouse FS20 broad spectrum UVB lamp was used. Case 6 is slightly more sensitive, but the remaining three XP-As and the two XP-Cs show similar sensitivity. Sensitivity to UVC killing varied from 5 to 11 times the normal.

Unscheduled DNA synthesis (an indicator of excision repair) following 5 J/m^2 UVC irradiation was measured for six XP-As (all save case 14) and the two XP-Cs. XP-A cases 12 and 13, and the XP-C cases appeared to show greater residual repair.

Persistence of inhibition of RNA synthesis 24 h after the irradiation with 5 J/m² of UVC was measured for two XP-As (Cases 6 and 12) and one XP-C (Case 8). RNA synthesis was inhibited in the two XP-As, but had recovered normally in the XP-C. RNA synthesis was inhibited 16 h after the irradiation with 10 J/m² in the cases 14 and 15.

It proved possible to generate short-term keratinocyte cultures from five of the biopsies (XP-A cases 5,6 and 12; XP-C cases 8 and 10) and to perform limited studies on UVB-induced apoptosis and cytokine release. These data have been reported previously (Petit-Frere et al, 2000), and are

summarised in Table 7. All three XP-A cases showed substantially greater apoptosis that the normal keratinocyte controls. However, the two XP-C cases gave different responses. XP-C case 8 gave an XP-A-like apoptotic response, whereas XP-C case 10 showed the intermediate level of apoptosis which might have been anticipated (Ljungman and Zhang, 1996). Only for the three XP-A cases could the keratinocytes be tested for sensitivity to UVB-induced cytokine release. All showed increased release. The unirradiated cultures also showed increased release of interleukin-6, but not tumour necrosis factor-α.

Discussion

We identified patients in three different XP groups (XP-A, XP-C and XP-G) in Finland. In most XP patients the features can be attributed to defects in NER of DNA damage. There are two sub-pathways of NER, transcription-coupled repair (TCR), in which damage in the transcribed strand of active genes is rapidly repaired, and global genome repair (GGR), a slower process for removing damage from the bulk of the genome. XP-A and XP-G patients are defective in both these processes, whereas XP-C patients are deficient in GGR but proficient in TCR.

XP group A is the most frequently occurring type worldwide, as is also the case in Finland. The neurological abnormalities in most of our patients suggest that our XP-A patients are early juvenile onset type, resembling the clinical features originally reported by De Sanctis and Caccione. These patients are clinically (neurological, cognitive, ocular and dermatological manifestations) quite similar in different ages, and indeed we found that they all had the same truncating mutation in exon 6. This mutation arg228ter, found in 6 out of 7 of our XP-A patients has been reported previously in several XP-A patients from Japan and Tunisia (see http://xpmutations.org/mutations.html). These cases were considered to show somewhat milder clinical manifestations than those of patients with a mutation affecting splicing of intron 3 which would be essentially a null mutation resulting in no functional protein (Nishigori et al., 1993; Maeda et al., 1995).

Case 6 is a late juvenile onset type showing only mild signs of cognitive impairment.. The mutation in this patient generates aberrantly spliced mRNA, which would not be expected to produce any functional protein. However we previously reported an XP-A case with no neurological abnormalities despite having barely detectable NER. This patient also had a splicing abnormality, but we were able to detect a very small amount of normal splicing to generate minimal levels of normal protein. It appeared that this small amount of normal protein was sufficient to allay all the neurological problems normally associated with XP-A defects (Sidwell et al., 2006). We suspect that the mild features of case 6 are similarly likely to result from a small amount of normal splicing of the mRNA. This patient showed immunosuppression in the cell-mediated immunity like the XP-C patients, however, she was not especially prone to skin malignancies (Table 2). As found in several other studies, our XP-C patients did not show any significant neurological abnormalities, indicating the importance of TCR in protection from lesions that might generate neurological damage.

All the patients demonstrated acute sun sensitivity in the first year of life. However, this led to diagnosis in only two of the cases. Despite the numerous skin malignancies, even melanomas, the XP-C patients have survived and some of the tumours seem to have self-healed. The phenomenon of spontaneous regression of primary and secondary melanomas in a XP-C patient has been described previously (Anstey et al., 1991) and it was proposed that the natural killer cell function led to the regression of the melanomas. The immunological status of XP needs further studies.

No diagnosis of internal malignancies was done in our XP patients during this follow up and the cause of death was pneumonia in all our patients. However, in the literature (Di Giovanna et al, 1998, Kraemer et al., 1987, Kraemer et al., 1994) there is a suggestion of an approximate ten- to twenty- fold increase in internal malignancies.

The eye findings of our patients were similar to those reported earlier (Dollfus et al., 2003, Goyal et al., 1994). Two patients with XP-C became permanently visually handicapped. The exposure to excessive sunlight perhaps caused the limbal premalignant dysplasia in a young man with XP-A working as a

gardener in a cemetery.

Neurological affision, including low intelligence, spasticity, ataxia, areflexia and hearing loss, is known to be associated with XP in about 18 per cent of cases published up to 1982 (Kraemer et al, 1987). In Japanese patients with XP-A over 7 years of age sensorineural deafness, nystagmus, dysarthria, ataxia and hyporeflexia were frequently observed (Mimaki et al., 1986). Both of above studies were cross-sectional and included mostly patients at early childhood. Because of a long follow-up period, we have been able to describe more precisely the natural course of this relentless disease. According to a previous knowledge we observed the most severe neurological disease in patients with XP-A (Thielmann et al., 1991, Rapin et al., 2000). However, we also noted neurological signs such as sensorineural hearing loss, neuropathy and laryngeal dystonia in XP-G patients thought to be free of neurological illness (Rapin et al., 2000). In XP-A patients, there is a certain order in which the neurological symptoms seem to appear. The neurological symptoms probably start slowly at about the age of two and are clearly seen at the age of 4-5 years manifesting cognitive and cerebellar signs. Afterwards the neurological symptoms slowly progress, finally leading to a premature death while affecting the whole nervous system as also seen in our neuropathological study of case 1 (Roytta and Anttinen, 1986).

The XP-A patients, except the late juvenile onset type, showed more often than described earlier moderate to severe progressing intellectual deterioration, but no specific impairment profiles were found.

Neuroimaging (CT and MRI) correlated with the severity of the neurological and cognitive impairment in XP-A patients. Surprisingly the XP-C patients, though neurologically and cognitively asymptomatic, had minimal general atrophy, slight cerebellar atrophy and white matter signal changes probably reflecting degeneration of neural tissue.

UV radiation penetrates only into the skin. Therefore the cause of the neurological disease cannot be the UV photoproducts that generate the clinical spectrum of disorders presented by the skin cancers and

corneal damage. A likely cause of the neurological problems is the accumulation of unrepaired oxidative DNA lesions in the brain, which result in progressive neuronal death. Suggested candidate lesions are cyclopurines (Kuraoka et al., 2000 Brooks 2007). However compared to the skin, the CNS is a much less tractable system for biochemical and cell biological studies, and our understanding of the origin of the neurological abnormalities in XP is still in its infancy. Since nowadays patients that are diagnosed early and well protected from sunlight suffer minimal skin problems and are living longer than in the past, neurological problems associated with XP are becoming of relatively greater importance in XP families and should be the focus of future research.

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Table 1: Basic characteristics and summary of clinical symptoms

Patient	**Cell Designation**	**Comple-mentation group**	**Onset of neurological symptoms**	**Ocular symptoms**	**First sunburn**	Onset and number of skin malignances
Case 5	XP5TUF	A	8 yr	+	6 mo	None
Case 6	XP1TUF	A	21 yr	+	3 weeks	34yr BCC x 1
Case 7	XP6TUF	A	4 yr	+	11 mo	In adolescence NMSC > 10 CMM x 1
Case 11	XP8TUF	A	7 yr	++	12 mo	12yr BCC x 10
Case 12	XP3TUF	A	4 yr	+	4 mo	11yr BCC x 10
Case 13	XP7TUF	A	3 yr	+	3 mo	None
Case 14	XP9TUF	A	None	None	2,5 mo	None
Case 1	Nd	A*	5 yr	None	6 mo	14 yr BCC x 2
Case 2	Nd	Unassigned[$]	4 yr	+	3 mo	None
Case 3	Nd	A*	4 yr	+	2 mo	None
Case 4	Nd	Unassigned[$]	6 yr	ND	10 mo	23 yr BCC x 2
Case 9	Nd	A*	4 yr	+	8 mo	None
Case 8	XP2TUF	C	None	+++	10 mo	6yr NMSC > 100 CMM x 8
Case 10	XP4TUF	C	None	+++	8 mo	4yr NMSC > 100

Case 15	XP1HEF	G	NA	ND	4 mo	None
Case 16	Nd	G*	NA	ND	8 mo	None

Table 1 continued.

A* = XP-A, based on consanguinity with assigned cases

G* = XP-G, based on consanguinity with assigned case

[$]These patients were not assigned to a complementation group.

Nd, not done

NA, not applicable

ND, not determined

BCC=basal cell carcinoma

CMM=cutaneous malignant melanoma

NMSC=nonmelanoma skin cancer

Cell designation is given in the laboratory and describes the origin of the cell cultures, TUF means Turku, Finland and HEF means Helsinki, Finland. The number is the order the cell cultures were performed.

Table 2: Sensitization with 40μg Diphenylcyclopropenone (DPCP): primary allergic response and response to different doses of the allergen in challenge (Nd = not done)

Patient	Cell Designation	Comple-mentation group	Primary allergic response	6.4 μg	3.2 μg	1.6 μg	0.8 μg	0.4 μg
Case 5	XP5TUF	A	Yes	Nd	++	++	++	+
Case 6	XP1TUF	A	No	-	-	-	-	-
Case 7	XP6TUF	A	Yes	Nd	Nd	Nd	Nd	Nd
Case 12	XP3TUF	A	Yes	Nd	+++	+++	++	+
Case 13	XP7TUF	A	Yes	Nd	Nd	Nd	++	+
Case 8	XP2TUF	C	No	-	-	-	-	-
Case 10	XP4TUF	C	No	-	-	-	-	-

Table 3. Age (yrs) at onset of neurological abnormalities

Definite XP-A

Patient/ birth year	Any neurological symptom	Cognitive dysfunction	Cerebellar involvement	Basal ganglia involvement	Mental symptoms	Hearing* (audiogram)	Peripheral neuropathy* (ENMG)	Corticospinal involvement*	Wheelchair / bedridden	Age of death
Case 5 1961	8	8	15	20	15	20 SNHL	20 ++	20 +	27/33	38
Case 6 1962	21	21	33	33	33	34 Normal	34 ++	-	-	NA
Case 7 1962	4	4	16	-	-	36 SNHL	36 +++	36 ++	38/40	NA
Case 11 1968	7	7	8	30	10	Nd	Nd	24 +	30/30	33
Case 12 1970	4	4	4	-	-	28 SNHL	28 +++	28 ++	-	NA
Case 13 1981	3	3	5	-	-	18 SNHL	18 +	18 -	-	NA

Table 3. Age (yrs) at onset of neurological abnormalities (continued)

Probable XP-A and unassigned cases

Patient/ birth year	Any neurological symptom	Cognitive dysfunction	Cerebellar involvement	Basal ganglia involvement	Mental symptoms	Hearing* (audiogram)	Peripheral neuropathy * (ENMG)	Corticospinal involvement*	Wheelchair / Bedridden	Age of death
Case 1 1949	5	5	5	-	11	Nd	32 +++	32 +++	25/28	32
Case 2 1952	4	7	4	18	18	30 SNHL	30 +++	30 ++	32/-	40
Case 3 1960	4	4	10	17	17	22 SNHL	22 ++	22 ++	26/28	32
Case 4 1960	6	6	12	26	7	Nd	26 ++	22 ++	26/-	29
Case 9 1964	4	4	4	-	17	17 SNHL	17 ++	17 -	NA	35

basal ganglia involment = involuntary movements

SNHL = sensorineural hearing loss

- no symptoms

* shows the age when examined and severity of symptoms(- none, + mild, ++ moderate and +++ severe)

Nd, not done

NA, not applicable

Table 4. Neuropsychological findings

Definite XP-A

Patient	Age (yr) when examined	Impairment of intellectual function		
		Degree of overall impairment	**Relatively preserved cognitive domains**	**Most affected cognitive domains**
Case 5	20	Moderate to severe V IQ 70, PIQ 42	WM, semantic knowledge	Numerical reasoning, visuomotor skills, speech production limited
Case 6	19	None to mild VIQ 93, PIQ 100	Verbal skills, semantic knowledge, visuomotor skills	Verbal WM
	34	None to mild VIQ 86, PIQ 107	Verbal skills, semantic knowledge, visuomotor skills	Verbal WM Numerical reasoning
Case 7	36	Severe VIQ 44, PIQ 37		WM, visuomotor skills Speech production very limited
Case 11	30	Severe		
Case 12	28	Severe VIQ 47, PIQ 43	Visuoconstructive skills	WM, verbal skills
Case 13	18	Severe VIQ 49 PIQ -		WM Verbal and visuomotor skills
Case 14		Not done		

Table 4. Neuropsychological findings (continued)

Probable XP-A and unassigned cases

Patient	Age (yr) when examined	Impairment of intellectual function		
		Degree of overall impairment	**Relatively preserved cognitive domains**	**Most affected cognitive domains**
Case 1	32	Severe VIQ -, PIQ 30		
Case 2	30	Severe VIQ 58, PIQ 37	WM Semantic knowledge	Visuomotor skills Speech production limited
Case 3	20	Severe VIQ 45, PIQ 40	Selective visuoconstructive performance	WM, verbal skills Speech production very limited
Case 4§	13	Moderate		
	26	Severe		
Case 9	17	Moderate VIQ 76, PIQ 56	WM, semantic knowledge Speech production	Numerical reasoning

§=psychological examination/ behavioural evaluation

VIQ = verbal IQ

PIQ = performance IQ

WM = working memory

Table 5. Neuroradiological findings

Definite XP-A

Patient	Age(yr) when examined	Brain CT/MRI
Case 5	20	CT: Moderate cerebral, slight brainstem and cerebellar atrophy
	34	CT: Moderate general atrophy
Case 6	21	CT: Normal
	34	MRI: Calvarial hyperostosis, moderate general brain atrophy, moderate cerebellar and hippocampal atrophy, white matter T2-hyperintensity excluding U-fibers
	41	MRI: Increased calvarial hyperostosis, moderate general brain atrophy, severe atrophy in corpus callosum, basal ganglia and cerebellum; white matter hyperintensity slightly increased
Case 7	36	MRI: Calvarial hyperostosis; moderate general atrophy, severe atrophy in frontal and parietal lobes and corpus callosum, moderate hippocampal atrophy; general white matter T2-hyperintensity including U-fibers, basal ganglia T2-hypointensity
Case 11		Nd
Case 12	27	MRI: Calvarial hyperostosis, moderate general brain atrophy, moderate hippocampal and cerebellar atrophy; white matter T2-hyperintensity excluding U-fibers
Case 13	17	MRI: Hyperostosis in sphenoidal and temporal bones; slight general brain atrophy, slight hippocampal atrophy, moderate callosal atrophy; periventicular T2-hyperintensity
Case 14		Nd

Table 5. Neuroradiological findings (continued)

Probable XP-A and unassigned cases

Patient	Age(yr) when examined	Brain CT/MRI
Case 1	32	CT: Moderate central and cortical atrophy, prominent thickening of skull
Case 2	30	CT: Moderate central and cortical atrophy
Case 3	20	CT: Calvarial thickening, generalized cortical and central atrophy
Case 4		Nd
Case 9	17	CT: Moderate central atrophy, slight cortical and cerebellar atrophy

XP-C

Patient	Age(yr) when examined	Brain CT/MRI
Case 8	33	MRI: Minimal lobar cerebellar and brain stem atrophy, no hippocampal atrophy; slight periventricular and subinsular T2-hyperintensity
Case 10	32	MRI: Minimal general atrophy; slight cerebellar atrophy, minimal hippocampal atrophy; periventricular and subinsular T2-hyperintensity

Nd, not done

Table 6: Fibroblast data for individual cases

Patient	Cell Designation	Comple-mentation group and mutation	Cell survival (sensitivity x normal)[a]		Unscheduled DNA synthesis after UVC [b] (% vs normals)	Inhibition of RNA synthesis after UVC [c] (% vs normals)
			UVB	UVC		
Case 5	XP5TUF	A Arg228Ter	11 x	7.5 x	0 , < 3	
Case 6	XP1TUF	A G283A	22 x	11 x	0 , < 3	14.3
Case 7	XP6TUF	A Arg228Ter		10 x	2.4 , < 3	
Case 11	XP8TUF	A Arg228Ter		9 x	0 , < 3	
Case 12	XP3TUF	A Arg228Ter	14 x	10 x	27.0, < 5	20.4
Case 13	XP7TUF	A Arg228Ter	11 x	6.2 x	23.2, 20-25	
Case 14	XP9TUF	A Arg228Ter			< 3	< 10
Case 8	XP2TUF	C Nd	9 x	5.0 x	17.5 , 10-15	89.2
Case 10	XP4TUF	C Nd	11 x	5.5 x	13.1, 15-20	
Case 15	XP1HEF	G Nd		9 x	< 3	< 5
Fibroblast controls	1BR 48BR C5RO	normal	-	-	-	-

Notes: (a) Ratios of slopes of semilog survival plots (D_o or D10); (b) after doses of 5 and/or 16 J/m^2, respectively; (c) 24h after 5 J/m2 or 16h after 10 J/m^2

Table 7: Available keratinocyte data (summary of data presented in Petit-Frere et al, 2000)

Patient	Cell Design-ation	Comple-mentation group	% apoptosis at 150Jm^{-2} UVB	24h IL6 release (pg/ml) at 0 Jm^{-2}	24h IL6 release (pg/ml) at 100 Jm^{-2}	24h TNFα mRNA 100 Jm^{-2} normalised vs MC 0 Jm^{-2}	24h TNFα release (pg/ml) at 100 Jm^{-2}
Case 5	XP5TUF	A	24.1%	3125	6905	5.60	294.4
Case 6	XP1TUF	A	28.0%	1701	2483	87.2	19.4
Case 12	XP3TUF	A	22.6%	9826	10785	2.15	149.4
Case 8	XP2TUF	C	20.7%	-	-	-	-
Case 10	XP4TUF	C	8.3%	-	-	-	-
Keratino-cyte control	TE	normal	5.2%	-	-	-	-
Keratino-cyte control	WW	normal	1.8%	-	-	-	-
Keratino-cyte control	MC	normal	-	672	849	-	0
Keratino-cyte control	MM	normal	-	950	1076	1.37	0.1

Legends for the figures

Figure 1.

XP-C patient, absence of pigmentary changes is evident on the areas protected from sunlight.

Figure 2.

Ophthalmological findings in XP. Upper left: Pterygium. Upper right: Corneal clouding and conjunctival hyperemia. Bottom: Symblepharon and corneal clouding.

Figure 3.

XP-G patient

Figure 4.

White matter signal changes and atrophy in MRI of an XP-A patient.